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SPIROCHAETAL ANTIGENS IN THE SERUM DIAGNOSIS OF SYPHILIS*

By JOHN A. KOLMER, M.D.

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Antigens prepared of cultures of *Treponema pallidum* were first employed in complement fixation tests for syphilis by Noguchi (1), Craig and Nichols (2) and Kolmer, Laubaugh and Williams (3) about thirty years ago. At that time they were found inferior in sensitivity to alcoholic extracts of beef heart reinforced with cholesterol in the Wassermann test. In 1929, however, interest in spirochaetal antigens prepared of cultures of the Reiter strain of *Treponema pallidum* was greatly renewed by Gaehtgens and Otto (4). Since then a very large literature has accumulated on the use of this antigen (commercially produced in Germany under the name of "palligen") and other spirochaetal antigens which I have elsewhere recently reviewed (5).

Miss Kast, Mrs. Lynch and I (6) have found, however, that antigens prepared of cultures of *Treponema microdentium* and *Treponema macrodentium* are capable of fixing complement with

* From the Department of Bacteriology and Immunology, Temple University School of Medicine and the Research Institute of Cutaneous Medicine. Presented, by invitation, before the American Society of Medical Technologists, June 9, 1942.

syphilis reagin just as well as those prepared of cultures of various strains of *Treponema pallidum*, including the Reiter strain. In other words, complement fixation reactions with spirochaetal antigens in syphilis are of a group character and we have found the same to be true of agglutination of these spirochetes by normal and syphilitic sera (7). In fact Miss Kast and I suspect, on the basis of cultural characteristics, that the Reiter strain may not be *Treponema pallidum* at all.

Furthermore, it is now well proven that antigens prepared of cultures of alleged *Treponema pallidum* are capable of giving a varying percentage of nonspecific or falsely positive complement fixation reactions with the sera of nonsyphilitic individuals (6). In tests employing "palligen" these have varied from 0.4 to 3.4 per cent (5). In the Washington serologic survey, conducted by the Committee on the Evaluation of Serodiagnostic Tests for Syphilis, held in October, 1941, under the auspices of the U. S. Public Health Service (8), Miss Carola E. Richter, who kindly substituted for me, found that the Kolmer simplified tests conducted with an antigen of the Reiter strain prepared for me by Miss C. C. Kast at the Research Institute of Cutaneous Medicine, gave 6.3 per cent positive or doubtful reactions with the sera of normal individuals and nonsyphilitic patients, excluding those with leprosy and malaria. In that survey Dr. Eagle found 1.9 per cent positive or doubtful reactions in the same group with a spirochaetal antigen likewise prepared of the Reiter strain. With both antigens the percentage of these falsely positive reactions with the sera of nonsyphilitic individuals was higher in the case of those with febrile intercurrent illnesses, tuberculosis and malignant disease than in normal individuals.

Furthermore, they are especially likely to occur with the sera of individuals with malaria and particularly with leprosy, as shown by Kolmer, Kast and Lynch (1), Eagle and his colleagues (9, 10) and others, just as it is now well known that nonspecific or falsely positive Wassermann and flocculation reactions occur with a high percentage of the sera of individuals with these diseases. In the Washington Survey, Miss Richter reported 57.6 per cent positive or doubtful reactions with the sera of leprous patients tested by the Kolmer simplified method using tissue antigen (C.L.) and 27.1 per cent positive or doubtful reactions with my spirochaetal antigen,

while the Eagle Wassermann test gave 39.8 per cent and his spirochaetal antigen 20.6 per cent positive or doubtful reactions. It is quite evident, therefore, that the use of spirochaetal antigens does not identify as biologic false reactions the positive Wassermann and flocculation reactions observed in leprosy and malaria as Eagle and his associates (9, 10) thought might be possible.

In other words, I am now convinced that the sera of some nonsyphilitic human beings contain a natural spirochaetal antibody capable of yielding falsely positive complement fixation, as well as positive agglutination reactions, not only with suspensions of cultures of alleged *Treponema pallidum* but with other spirochetes as well with special reference to *Treponema microdentium* and *Treponema macrodentium*. Consequently, when spirochaetal antigens are used in the serum diagnosis of syphilis the dose employed should be carefully determined by titration with a mixture of syphilitic sera and the smallest permissible amount used in order to reduce to a minimum the incidence of these nonspecific or falsely positive reactions. For example, the antigen of the Reiter strain used by Miss Richter for me in the Washington survey had an anticomplementary unit of about 0.5 cc. of a 1:4 dilution in a titration by my method (11). The antigenic unit was 0.5 cc. of 1:40 dilution. The dose employed was 0.5 cc. of 1:20 dilution corresponding to two antigenic units or one-fifth the anticomplementary unit.

However, while this natural spirochaetal antibody may occur in sera, it does not occur in the spinal fluids of nonsyphilitic human beings (6). This is due to the well known fact that natural antibodies like the antitoxins, agglutinins, opsonins, etc., commonly present in normal sera, do not occur in normal spinal fluids. Consequently, and as I fully expected, both Miss Richter and Dr. Eagle observed no nonspecific or falsely positive complement fixation reactions with the spinal fluids of nonsyphilitic individuals tested with spirochaetal antigens in the Washington survey.

Undoubtedly spirochaetal antibody is increased not only in malaria and leprosy but in syphilis as well for antigens of spirochetes. Kolmer, Kast and Lynch (12) have found that this acquired antibody in syphilis is more likely to give positive complement fixation reactions with antigens prepared of virulent *Treponema pallida* (Nichols-Hough strain) recovered from acute testicu-

lar syphilomas of rabbits than with antigens prepared of cultures of nonvirulent *Treponema pallidum*, but because of the technical difficulties of securing the tissue spirochetes free of cellular debris, and in view of the small yield, it is not likely that antigens of virulent tissue spirochetes can be used on a large scale in the serum diagnosis of syphilis.

Furthermore, whether or not spirochetes contain a ubiquitous lipid also present in alcoholic extracts of beef heart with the result that the Wassermann, flocculation and spirochaetal complement fixation reactions are due to spirochaetal antibodies, as stated by Eagle and Hogan (13), is still undecided. On the basis of results observed in absorption experiments Kolmer, Kast and Lynch (7) concluded that the reagin responsible for the Wassermann and flocculation reactions in syphilis was entirely different from the antibody producing positive complement fixation reactions with spirochaetal antigens and apparently Gaehtgens, Kroò and his colleagues and Beck are of the same opinion (5).

Whether or not spirochaetal antigens prepared of cultures of the Reiter or other strains of alleged *Treponema pallidum* are of practical value in the serum diagnosis of syphilis by complement fixation is likewise undecided at the present time. In the Washington survey my simplified test conducted by Miss Richter with the tissue or lipoidal (C.L.) antigen had a sensitivity rating of 74.1 per cent and my spirochaetal antigen 70.6 per cent. The rating for the Eagle Wassermann test was 59.2 per cent and for his spirochaetal antigen 75.9 per cent. It will be observed, therefore, that my tissue or lipoidal antigen had a greater sensitivity than my spirochaetal antigen but that the Eagle spirochaetal antigen was more sensitive than his tissue or lopoidal antigen. Why this difference in the results occurred, I am unable to state and especially since both of the spirochaetal antigens were prepared of the Reiter strain. Gaehtgens has never revealed the details of the preparation of his "palligen" but from his papers one may surmize that it is supposed to be a suspension of whole spirochetes in phenolized saline solution. Consequently, Miss Kast and I have prepared our spirochaetal antigen of the Reiter strain as follows: A pure culture was cultivated in beef heart infusion broth (Difco) containing 0.05 per cent thioglycollate and 10 per cent sterile horse serum for five to seven days at 37° C.

in 125 cc. round-bottomed flasks with vaseline seals. (The sterile horse serum was first heated in a water bath at 63°-65° C. for 30 to 60 minutes and thoroughly centrifuged for the removal of precipitates before addition to the broth). The cultures were then centrifuged for one and one-half hour at approximately 3000 r.p.m. in a conical head International centrifuge and the sediment of spirochetes washed twice with large volumes of sterile saline solution. After the last washing the spirochetes were suspended in sufficient 0.5 per cent solutions of phenol in physiological saline solution to give a turbidity approximately equal to a reading of 450 on the Kleet-Summerson colorimeter. Upon completion the antigen was titrated for its anticomplementary and antigenic units, as previously stated, and stored in the refrigerator.

As previously stated, I believe that the reagin in syphilitic serum giving positive Wassermann and flocculation reactions with the lipoids in tissue antigens is separate and distinct from the antibody producing complement fixation and agglutination of spirochetes. Of course it is both possible and probable that the reagin also fixes some complement with the lipoids of spirochetes as I observed thirty years ago (3). Whether spirochaetal antibody reacts with these lipoids is unknown but the investigations of Beck (14), confirmed by Kolmer, Kast and Lynch (7), suggests that it fixes complement with some other constituent of spirochetes of a nonprotein nature. Indeed, the whole matter goes back to the early days of the Wassermann reaction when saline or alcoholic extracts of fetal syphilitic liver were preferred as antigens for the test. With this newer work one wonders if these older antigns may have contained not only the tissue lipoids for fixation of complement with syphilis reagin, but specific antigen of *T. pallidum* for fixation of complement by syphilis antibody as well.

With these considerations in mind I have had Mrs. Lynch test syphilitic and normal sera with mixtures of my tissue or C.L. and spirochaetal antigens. We have observed that a mixture of equal parts of 1:300 dilutions of C.L. antigen and 1:10 dilution of spirochaetal antigen in dose of 0.5 cc. gives better results in the regular Kolmer simplified test than spirochaetal antigen alone in dose of 0.5 cc. of 1:20 dilution. Sensitivity has been found to be appreciably increased although the reactions observed with syphilitic sera were

not as sensitive as with the tissue or lipoidal (C.L.) antigen alone in dose of 0.5 cc. of 1:600 dilution. In other words, the mixture of the two antigens in some manner decreases the sensitivity of the tissue antigen but yet renders the mixture more sensitive than the spirochaetal antigen alone in dose of 0.5 cc. of 1:20 dilution. Furthermore, quite unexpectedly, and for reasons as yet unknown, the mixture of the two antigens has given a smaller percentage of nonspecific or falsely positive reactions with normal sera than the spirochaetal antigen alone. Under the circumstances it may be, therefore, that a mixture of the two antigens may prove more satisfactory in the serum diagnosis of syphilis than the spirochaetal antigen alone although, in my experience, spirochaetal antigens alone or in the mixture have not proven as sensitive or as specific in the Kolmer complement fixation tests for syphilis as the tissue or lipoidal antigen (C.L.) when used in its optimum dose.

Conclusions

1. Complement fixation in syphilis with spirochaetal antigens is a group reaction as positive reactions occur with antigens prepared not only of cultures of the Reiter and other strains of *T. pallidum* but of *T. microdentium* and *T. macrodentium* as well.

2. Antigens prepared of cultures of these spirochetes give a small and varying percentage of nonspecific or falsely positive complement fixation reactions with the sera of normal individuals and those with tuberculosis, malignant disease and febrile intercurrent illnesses ascribed to the presence of natural spirochaetal antibody.

3. Spirochaetal antigens give a particularly high percentage of nonspecific or falsely positive complement fixation reactions in leprosy and malaria just as tissue or lipoidal antigens yield a high percentage of positive Wassermann and flocculation reactions in these diseases.

4. The spinal fluids of nonsyphilitic individuals, however, do not give nonspecific or falsely positive complement fixation reactions with spirochaetal antigens which is ascribed to the absence of natural spirochaetal antibody in them.

5. Spirochaetal antigens prepared of virulent *T. pallidum* recovered from acute testicular syphilomas of rabbits give a higher percentage of positive complement fixation reactions with syphilitic sera and a lower percentage of falsely positive reactions with normal sera than antigens prepared of cultures of alleged *T. pallidum*.

6. Available evidence, based upon the results of absorption tests, indicates that the syphilis reagin giving positive Wassermann and flocculation reactions is entirely separate and distinct from the natural spirochaetal antibody in normal sera or the acquired spirochaetal antibody in syphilis yielding positive complement fixation reactions with spirochaetal antigens.

7. The value of spirochaetal antigens prepared of the Reiter strain in the serum diagnosis of syphilis is as yet undecided. In the Washington serologic survey the Kolmer spirochaetal antigen was less sensitive than his tissue or lipoidal extract (C.L.). The Eagle Wassermann reaction using tissue antigen, however, was less sensitive than a spirochaetal antigen.

8. A mixture of spirochaetal and tissue or lipoidal antigens has been found more sensitive in complement fixation tests with syphilitic sera than spirochaetal antigen alone but less sensitive than tissue or lipoidal antigen alone. The mixture has also given a smaller percentage of nonspecific or falsely positive reactions with normal sera than the spirochaetal antigen alone.

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A DEVICE FOR READING THE KAHN TEST*

By HINTON F. MILLER

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The normal human eye, unaided by magnifying devices, can readily discern the precipitate in a positive Kahn reaction; but there is no disputing the fact that two or three power magnification enables the average eye to see this precipitate better. Quoting the Report of the 1930 Serological Conference of the League of Nations Health Organization:

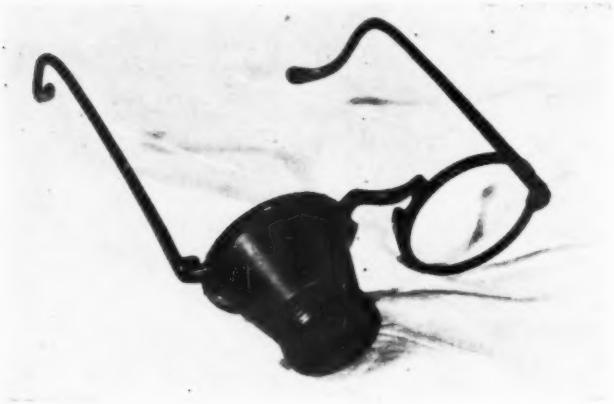
"Those preferring magnification will find the microscopic mirror helpful. Place mirror on reading table with concave surface upward. Hold the tube in slanting position two or three inches above the mirror and examine the image in the mirror. Both daylight and artificial light may be employed. One may also utilize an ordinary hand lens for reading the tests. A two- or threefold magnification will be found satisfactory. Some workers prefer the use of a slit-bulb arrangement, the source of light being an electric bulb enclosed in a box which is provided with a narrow slit.

"As far as possible, workers should limit themselves to one method of reading. The occasional use of magnification by readers who do not usually resort to it will be likely to affect the uniformity of their reading scale. It should be emphasized that certain highly magnifying agglutinoscopes show particles in serum alone, and are thus unfit for use in the tests. The magnification must be sufficiently low as to assure opalescent and clear cut negative reactions, with entire freedom from visible particles."

In reading a large number of Kahn tests, the use of the concave mirror from a microscope slows the interpretation greatly, as each tube has to be lifted from the rack and inspected individually. The

* From the U. S. Public Health Service Laboratory of the U. S. Marine Hospital, Seattle, Wash.

hand lens is somewhat clumsy, as it leaves only one hand free to manage the test-tube rack.



The magnifying device shown in the illustration leaves both hands free for handling the rack and recording results of the test on a notepad. It was constructed as follows: an optical eyepiece, or "jeweler's glass" was purchased. (The one shown was obtained second-hand, cost fifty cents. It is two power, has a focal length of five inches). Next a pair of cheap sun glasses, costing about 25c and having a lens frame diameter to fit the jeweler's glass, was obtained and the dark glass removed from both eyepieces. The jeweler's glass was fastened neatly in one eyepiece of the spectacle frame with adhesive cellulose tape.

In using the magnifying eyepiece, the slit-bulb arrangement is convenient. A slit 1½ inches wide and 6 inches long may be cut in the laboratory bench and a frosted light bulb placed beneath it. The Kahn rack and tubes to be inspected are tilted over this slit with the light beneath and examined from above with the eyepiece. A box with a slightly slanting top and slit may also be used as a light source. During the reading, any bright extraneous source of light

that makes the light from the slit appear relatively dimmer should be cut off.

A large number of Kahn tests may be read rapidly and accurately with the device shown, as it is unnecessary to remove the tubes from the rack for examination. A similar magnifying eyepiece mounted in spectacle frames may be purchased from an optical supply house, as Bausch and Lomb, but the cost is much higher.

METHODS FOR DEMONSTRATING TUBERCLE BACILLI IN SPUTUM AND OTHER MATERIALS*

By JOSEPH M. SCOTT, M.T. (ASCP)

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I propose to discuss some of the methods that may be used to demonstrate the tubercle bacillus in sputum and other materials. Some are comparatively simple and easily applied in a small laboratory, while others are more complicated and better suited to a sanatorium laboratory. The actual techniques I will discuss are by no means the only ones, and not necessarily the best, but rather some that have been used with success in one sanatorium laboratory. I do not intend to give you every detail of these techniques, for it would take too long, and most of the information can be readily found in various textbooks, but rather I wish to speak more of the general application of the various methods.

Of methods in general it might be said that the more refined they become the harder it is to get tuberculous patients bacilli free, especially their sputum, an observation that has been made in more than one sanatorium. But there is still room for improvement, as can be seen by the very variable results obtained in different laboratories.

Tuberculosis is still one of the most prevalent and serious of diseases, in spite of widespread anti-tuberculosis activities, and almost every medical technologist will be called upon at some time or another to make examinations for the tubercle bacillus. The methods used for sputum will receive the greatest emphasis since the pulmonary form of the disease is the commonest.

I need not remind you of the complexities of bacteriological identification. Fortunately the tubercle bacillus has certain definite

* *Presented at the Annual Convention of the American Society of Medical Technologists, Philadelphia, June 8, 1942.*

characteristics, notably its acid-fast properties and its slow rate of growth, which make its recognition relatively easy, but unfortunately there are other acid-fast bacilli which might lead to errors in identification. Such organisms have been found in tap water, old distilled water, butter, the wax of the ear, and especially in the secretions about the genitals. Some methods for avoiding or excluding such organisms will be given later.

Methods of Examination

The methods of examination for the tubercle bacillus may be divided into four main classes as follows:

1. Direct smear.
2. Concentration.
3. Culture.
4. Animal inoculation.

The direct smear method is the simplest and commonest one used and the result can be known within 30 minutes. It is applicable to all materials, especially sputum, and if a quick report is imperative it is the method of choice. Furthermore it is a basic method since it is necessary for the final microscopic proof of tuberculosis whether concentrates, cultures, or animals are used. Many positive results will be missed by this method, but its use is justifiable in every instance. In the case of sputum repeated examinations are more valuable than single ones; and 24-hour specimens better than random ones.

A concentration method may require 24 hours for the report and will frequently demonstrate bacilli in specimens found negative by direct smear. There are many different methods but not all are as satisfactory as could be desired. Some require more time than others but often give the best results. Concentration methods have their greatest usefulness in tuberculosis institutions.

Cultural methods are very important in tuberculosis for the results obtained are more dependable than those obtained from smears or concentrates. Since the tubercle bacillus grows slowly the results are not known for two or three weeks, and oftener it is four to six. Cultures may be used for all kinds of material and are particularly

valuable for puncture fluids. If used consistently the number of positives will be greatly increased. Non-pathogenic acid-fast organisms can usually be differentiated from the tubercle bacillus by culture.

Most authorities are agreed that the inoculation of guinea pigs is the most reliable laboratory test we have for the diagnosis of tuberculosis. The long wait required for the results, usually two to three months, is a decided disadvantage but is not so objectionable if the patients are on treatment. In many cases the reliability of the report more than justifies the delay. In genito-urinary tuberculosis the guinea pig is of great value since it will differentiate between the tubercle bacillus and the smegma bacillus, an acid-fast organism found on the skin and in the secretions about the genitalia, and a likely contaminator of urine. If urine is positive by both culture and guinea pig one can be certain of a diagnosis of tuberculosis. Guinea pigs are more expensive to keep than most culture media are to prepare, and many small laboratories may not have them at all.

Some investigators maintain that they obtain more positive results from cultures than from animal inoculation, but mostly the reverse is true. In 1932 Magath and Feldman (1) published an excellent comparison of results obtained by both methods at the Mayo Clinic and definitely favored the guinea pig as the most reliable.

Direct Smear Method

There are a number of different staining methods for the tubercle bacillus but the best known is that of Ziehl-Neelsen which has been used for over 50 years with few, if any, essential changes.

In 1926 Cooper (2) introduced a modification of this method which we have used for many years and found very satisfactory. To the ordinary Ziehl-Neelsen stain 10 per cent sodium chloride solution is added to the extent of 3 per cent, the effect being to cause the stain, when it cools, to precipitate upon the bacilli, thus resulting in a better staining reaction. As a decolorizer 5 per cent nitric acid in 95 per cent alcohol is used, with 1 per cent brilliant green as the counterstain.

In the examination of smears a good microscope is essential. If many slides are to be examined daily a binocular type is desirable as

it will prevent much eye strain. A magnification of X 1000 is satisfactory but X 1500 is better. We believe that slides counterstained with brilliant green are more easily examined than those stained with methylene blue or picric acid, although this may be simply a matter of individual fancy. The best slides are those that are not too thick, since it is difficult to recognize acid-fast bacilli with certainty in the thicker parts, but on the other hand if too thin the examination becomes tedious and unsatisfactory.

In our laboratory routine sputum slides are examined for three to five minutes before being called negative. More time is given to slides of materials more difficult to obtain such as gastric contents or cerebrospinal fluid. If tubercle bacilli are plentiful it is comparatively easy to identify them, but when only one or two are found after prolonged search caution must be used in rendering a positive report, especially if it is the first examination made for the patient. Unless very clear-cut and characteristic at least four or five bacilli should be found before making a positive report. In cases of doubt it is wise to have another technologist check your findings. Todd and Sanford (3) advise that if there is any doubt about an acid-fast bacillus seen in a smear the structure in question is probably not a tubercle bacillus.

Mention might be made here of a new method for demonstrating tubercle bacilli that holds promise for the future—that of the fluorescent dye auramine and ultraviolet light. A favorable report has recently come from Thompson (4) of the Mayo Clinic.

Concentration Methods

Most of the concentration methods are intended primarily for sputum but some may be adopted to other materials as well.

For a number of years we used Sweany's (5) sodium hydroxide concentration method for sputum but with variable degrees of success. We found it difficult to make satisfactory smears due to the fact that materials treated with sodium hydroxide do not stick very well to slides, even when albumen fixative is used.

More recently we have used the dilution-flotation method, employing a liquid hydrocarbon, for concentrating sputum. This method was first devised over 30 years ago by Lange and Nitsche

but was improved and popularized in later years by Pottenger and Smith (6) of California. It requires more time to carry out than Sweany's method but in our hands has yielded better results.

By this method sputum, collected over a 24-hour period, is put into a wide-mouthed 6-ounce bottle with an equal amount of 0.5 per cent sodium hydroxide solution and shaken in a mechanical shaker for 15 minutes. After standing for 30 minutes in a water bath at about 56 degrees C. the specimen is diluted with distilled water and 1 cc. of xylol added and shaken for another 15 minutes. The bottle is then stood upright for two to four hours during which time the xylol collects on the top of the fluid to form a cream-like layer, bringing many tubercle bacilli with it. This creamy layer is taken up in a dropper pipette, allowed to stand upright for further separation and the "cream" placed on a slide in an oven at about 56 degrees C. As the fluid evaporates away five or six more layers are added. The dry smear, which requires no further fixation, is washed in ether and stained in the usual way for sputum. Good slides are thin and clean and the tubercle bacilli stain well and, though often rather beaded, are usually easily identified.

The method we use for the treatment of gastric contents is essentially a concentration method, and is the one given by Ulmar and Ornstein (7) of New York. The material is thoroughly mixed with 3 per cent sodium hydroxide solution in 50-cc. centrifuge tubes to dissolve mucoid material, and after standing for 15 minutes is centrifuged at high speed for 15 minutes. Smears are made of the sediment, using an albumen fixative, but the same difficulty is encountered here as in the Sweany method for sputum. They should be made moderately thick and washed very carefully when being stained.

A surprisingly large number of positive results will be encountered in examining gastric contents since sputum is so frequently swallowed. Macklin and Wales (8) found that many more positives were obtained if cultures were also used, while Roper and Ordway (9) found the same true when guinea pigs were similarly used.

Cultural Methods

Success in the cultivation of the tubercle bacillus depends to a

considerable degree on the quality of culture media being used. Many kinds have been developed for this purpose and names like Dorset, Loewenstein, Petroff, Corper, Herrold and Petragnani are familiar to most technologists. Some of these media are difficult to prepare in small laboratories because of the lack of certain essential equipment, such as a good inspissating oven or an Arnold sterilizer. In certain cases where the amount of culturing done is not very extensive good media may be obtained already prepared in culture tubes, but the cost is much higher than for that of media prepared in the laboratory.

For a small laboratory Corper's (10) crystal violet potato medium or Herrold's (11) egg yolk broth agar medium are very satisfactory and both can be easily prepared if an autoclave is available. In recent years there has been great interest in Petragnani's medium and my own experience with it, though rather limited, is that it is on of the best for the primary cultivation of tubercle bacilli. It is, however, more difficult to prepare than the other two. Macklin and Wales (8) well describe its preparation.

I believe it is a good practice to use at least two different media, inoculating a tube of each for every specimen cultured, or if only one is available, two tubes for every specimen.

For destroying contaminating bacteria in sputum and urine we have used both 6 per cent sulphuric acid and 5 per cent oxalic acid but believe the latter is the more satisfactory. Puncture fluids, if handled properly, require no preliminary treatment before culturing, other than centrifugation if they are clear or only slightly cloudy.

Animal Inoculation Method

The collection of urine specimens in sterile containers so as to avoid contamination from other sources is an important requirement, particularly in cultural and guinea pig work, and is a precaution that is sometimes overlooked. It is particularly necessary in an institution where containers are used repeatedly and where there are cases of genito-urinary tuberculosis known to be discharging tubercle bacilli into the urine. This source of error may be largely avoided if only urine secured by catheterization, especially from women, is used for inoculation purposes.

It is very important, and certainly more humane, to treat the urine before inoculation to kill contaminating organisms. After collecting the sediment from about 100 cc. of urine by centrifuging it at high speed in 50-cc. centrifuge tubes it is treated with about 2 cc. of 3 per cent hydrochloric acid solution, then neutralized with 3 per cent sodium hydroxide, using phenolphthalein as the indicator. Since we began using this treatment I cannot recall a guinea pig having had an abscess or open sore at the site of inoculation.

The inoculated pigs are killed after about 12 weeks unless they have died before that time. A careful autopsy is done in every case and smears are made of material from the lesions in each diseased organ such as spleen, liver, lungs, lymph nodes, etc. Special search should be made for enlarged glands which have become abscessed or caseous. Tubercle bacilli may often be found more easily in pus from abscessed glands than from lesions in other organs. Material from other lesions may be crushed between two slides, fixed, and stained in the ordinary way. If the tuberculosis is well advanced tubercle bacilli can usually be found, though they may not be very plentiful. We do not prepare histological sections for the diagnosis of tuberculosis in the guinea pig.

Examination of Various Materials

Let me say a few words now about materials that are likely to be examined much less frequently than sputum or urine.

Cerebrospinal fluid is one of the most difficult materials in which to find tubercle bacilli. In tuberculous meningitis a pellicle usually forms in the fluid after it stands in a cool place for several hours. It should be floated out onto a slide, fixed, stained, and carefully and completely examined. The results are most often negative. Cultures and guinea pigs may be used but the patient has usually expired before the results are known.

Pus may be treated in the same manner as pleural fluids, which is true of other puncture fluids, such as ascitic, pericardial and hydrocele.

Feces are not often examined for tubercle bacilli, especially since the examination of gastric contents has become much more common.

Conclusion

From what has been said you can see that there are many methods to choose from in the laboratory study of tuberculosis. If you cannot use all of them because of the limitations of your laboratory facilities, or the type of work you are called upon to do, adopt those that seem most suited to your circumstances and become as proficient as possible in their use.

Do not be discouraged if many, or most, of your results are negative, for that is the experience of those who work in the laboratories of tuberculosis hospitals. Your greatest satisfaction should come from knowing that the results of your work, whether negative or positive, have helped in the diagnosis of more than one difficult case.

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LABORATORY PROCEDURES IN THE DIAGNOSIS OF MALARIA

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The most conclusive laboratory procedure in the diagnosis of malaria is the demonstration of the malarial parasites in the red corpuscles. During an acute attack of untreated malaria, parasites are often readily demonstrable in thin smears stained with Wright's stain. When the acute attack has subsided, or when treatment has been begun, the parasites become very rare or may disappear altogether. Under these circumstances the thick film stained with Giemsa's stain is used. The parasites are found lying free and particular study is required to identify them positively.

A number of associated abnormalities may be observed in the blood in malaria. A pronounced reticulocytosis often follows adequate treatment of the disease; a sustained reticulocytosis characterizes a persisting infection. There are also poikilocytosis, anisocytosis, polychromasia and stippling in the chronic forms of malaria. The red cells in vivax malaria may show a fine red stippling; these are called Schuffner's dots and occur usually in parasitized cells. In falciparum malaria parasitised cells may show coarse violet dots, Maurer's dots, which are quite diagnostic of the infection when found.

The anemia, even in the pernicious forms, is often not severe. Leucocytosis is common during a paroxysm, but leucopenia with a relative monocytosis characterizes the afebrile periods. If leucocytes are found carrying pigment the suspicion of malaria is greatly strengethened; however this finding is not a common one.

There are several other laboratory procedures for the diagnosis of malaria. With the exception of the complement fixation test and the agglutination and protection tests, the procedures are nonspecific.

A. The Complement Fixation Reaction.

Coggeshall and Eaton found this test to be specific for malaria and adapted its use to the diagnosis of malaria. It is a group-specific reaction, meaning that antigens from one species will react with positive sera from other species. It has been applied to the diagnosis of human malaria with a good deal of success. A positive outcome may be accepted as proof of malarial infection, but a negative result does not exclude it. The test becomes positive in about three or four weeks and tends to become negative again in three or four months after infection.

B. The Agglutination Test.

Immune sera will agglutinate living parasites. This phenomenon has not been brought into clinical laboratory use. It is a highly specific reaction.

C. The Protection Test.

Immune serum will protect laboratory animals against severe attacks. Likewise, incubation of living parasites with immune serum will disinfect them. However, both these procedures have not been applied clinically.

D. Non-Specific Procedures.

A marked increase in the euglobulin of the blood is characteristic of acute malaria. There are a number of reactions designed to react positively under these circumstances. The reactions are non-specific and will react positively in those diseases having an increase in the euglobulin of the blood, namely, leprosy, active tuberculosis, typhoid fever, granuloma inguinale and the like.

1. *Henry's Melanoflocculation Test*

In 1927, Henry showed that the blood serum of malarial subjects will flocculate if mixed with a suspension of melanin taken from bulls' eyes. It has since been shown that simply mixing the serum with water will bring about a similar flocculation. The addition of melanin makes the reaction measurable with the naked eye. The efficiency of the test is still under debate; however, it turns out positively in malaria in a high percentage of cases.

2. *The Protein Tyrosine Reaction of Proske and Watson*

This reaction is essentially a determination of the euglobulin based on the colorimetric estimation of tyrosine contained in a sodium sulphate precipitate of serum. The test is positive in malaria in a good percentage, but is also positive in the diseases mentioned above.

The laboratory diagnosis of chronic malaria is difficult. Often it is important to learn whether a person is suffering from a malarial infection of any sort when blood preparations are negative. For example, bizarre chronic disease of great complexity may be caused by malaria, and proper diagnosis and treatment would be highly desirable. The recognition of a latent infection is important in blood donors. Acute malaria which has been improperly treated represents another condition in which a good diagnostic test would be useful. It becomes obvious, therefore, that any test which can *exclude* malaria is useful. Unfortunately, there is no such test. However, the tests enumerated, when positive, should help to direct attention to a possible malarial condition.

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ABSTRACTS

THE SIGNIFICANCE OF URINARY CALCIUM, MAGNESIUM AND PHOSPHORUS: R. A. McCance & E. M. Widdowson. Jr. Physiol., vol. 101, No. 3, Nov., '42, p. 350.

Urinary excretion of calcium, magnesium and phosphorus by normal persons was found to rise and fall with the amounts absorbed from the intestine. They did not, however, vary over as wide a range as the absorption.

The authors point out that the function of the kidney in this respect is to regulate the stability of the internal environment. Small increases in the amount of calcium, magnesium and phosphorus entering the blood serum are taken care of by the kidney alone. If the amounts are larger, deposition occurs in the skeleton. In some diseases, as in increased parathyroid activity, calcium is taken from the skeleton and the kidney, normally in balance with the skeleton, is unable to take care of the excess and the serum calcium rises.

In thyrotoxicosis and myxoedema the process is reversed. The kidney's excretion of calcium is increased, the blood serum level drops and decalcification of the skeleton occurs.

THE ANTIBACTERIAL ACTION OF CERTAIN DISINFECTANTS: A. Hoyt, R. T. Fisk & G. Burde. Surgery, vol. 12, No. 5, Nov., '42, p. 786.

Disinfectants commonly used for the cold sterilization of skin and instruments were tested for their bacteriostatic and bacteriocidal action against S. aureus and B. coli at room temperature. Mercurial preparations showed high bacteriostatic activity but when subcultured in broth or when cultured in sodium thioglycolate broth, they showed relatively low bacteriocidal activity.

Iodine solutions and Zephiran showed good bacteriocidal activity and their use and limitations should be investigated further.

PRONOUNCED AZOTEMIA: REPORT OF TWO CASES: E. L. Walsh. Med. Bull. Vet. Adm., vol. 19, No. 2, Oct., '42, p. 208.

Two cases of polycystic kidneys are reported in detail. Both retained consciousness until the terminal phase and both had N.P.N. readings of 600 mgs.% on the day of death.

The author suggests that when the rise in N.P.N. is gradual, the body is able to adjust to it, while in conditions in which the rise is sudden, coma quickly results.

THE TREATMENT OF FIFTY-SIX MENINGOCOCCUS CARRIERS WITH CHEMOTHERAPY: P. S. Strong & J. M. Blumberg. Military Surg., vol. 92, No. 1, Jan., '43, p. 59.

In the method used for these examinations a platinum loop was bent at an angle of 100° leaving about 4-5 cm. from the bend to the loop. The sterile loop was passed to the side of the uvula and into the posterior pharynx. It was inserted as high as possible into the nasopharynx and rotated slightly. If necessary, a tongue depressor was used to make the patient gag. The wire was carefully withdrawn to avoid contamination. Human blood agar was streaked and incubated at 37.5°C. in a "candle jar" under reduced oxygen tension.

After 24 hours, suspicious colonies were tested for the oxydase reaction by Carpenter's method. Oxydase positive colonies were Gram stained and subcultures made. After 24 hrs. bacterial suspensions were made from the subcultures and agglutination reactions using normal horse serum and polyvalent antimeningococcus horse serum. Only those showing agglutination with antimeningococcic serum in a dilution of 1-3200 and none with normal horse serum were considered positive.

Sulfathiazole (6 grams daily for 5 days) was found 100% satisfactory for the cure of 35 Meningococcus carriers.

BIOLOGIC FALSE POSITIVE TESTS FOR SYPHILIS ASSOCIATED WITH ROUTINE ARMY IMMUNIZATIONS: R. D. Arthur, J. M. Hale. Military Surg., vol. 92, No. 1, Jan., '43, p. 53.

The possibility of false positive Wassermann and Kahn tests due to immunization treatment was investigated. The subjects were soldiers receiving the usual rapid course of smallpox, typhoid and

yellow fever vaccines and 3 tetanus toxoid injections.

Of the 94 cases studied, 14 showed temporary changes in the normal negative Kahn reaction. These false positive reactions faded in 1-2 months.

As it was not feasible to delay the immunizing treatment, the authors could not determine whether the reaction was due to any particular immunization or to the combination given in such rapid succession.

A DIRECT COLORIMETRIC METHOD FOR THE DETERMINATION OF UREA IN BLOOD AND URINE: A. A. Ormsby. Jr. Biol. Chem., vol. 146, No. 2, Dec., '42, p. 595.

A direct method applicable to blood filtrate and to urine is described. Ammonia does not interfere. The author reports results whose accuracy compared well with that obtained by the common published procedures.

Technique:

The volume employed should contain preferably from 0.1 to 0.2 mg. urea (extreme limits, 0.05 to 0.3 mg.) and must not exceed 3 cc. For most urine samples this would be 1 cc. of a 1-100 dil. and for blood 3 cc. of Folin-Wu filtrate.

Place the material being tested in a test tube and make up to 3 cc. with distilled water.

Prepare 2 standards using 1 and 2 cc. of standard solution (1 cc. = 0.1 mg. urea plus a little chloroform as a preservative) and making up to 3 cc. with distilled water.

To each tube add 5 cc. conc. HCl and 0.5 cc. 3% biacetyl monoxime.

Mix by rotation and immerse in actively boiling water exactly 10 min. As it is necessary to prevent evaporation during heating, the tubes may be covered with funnels or glass marbles.

Remove simultaneously and cool in running water 2 min.

Add slowly 0.25 cc. of a 1% potassium persulfate allowing it to form a separate layer. After all tubes have been treated, stopper and mix by inverting a few times.

As the maximum color development depends upon the amount of urea present, readings are taken at 5, 15 and 25 min. Readings may

be taken in the Klett photoelectric colorimeter with a No. 42 filter or in a visual colorimeter.

The persulfate solution is refrigerated and made up fresh each week. New lots did not effect the curve readings. A new curve had to be made for each solution of biacetyl monoxime but this reagent is stable and can be made in large quantities.

DETERMINATION OF CHLORIDES IN BIOLOGICAL FLUIDS BY THE USE OF ADSORPTION INDICATORS. A NEW METHOD FOR THE DETERMINATION OF THE PLASMA VOLUME OF BLOOD: A. Saifer, J. Hughes and E. Weiss. Jr. Biol. Chem., vol. 146, No. 2, Dec., '42, p. 527.

Determination of the plasma volume is based upon the fact that when whole blood is dropped into a mixture of absolute alcohol and anhydrous ether (3:1) in such a way that the cells are not ruptured, the chloride ions do not pass through the cell wall. The chloride content of both plasma and whole blood plasma is determined by titrating with silver nitrate using dichlorofluorescein indicator.

A micromethod is also described by which duplicate readings may be obtained on less than 2 cc. oxalated blood.

This technique gave an average deviation of only 1% from the results obtained by the Evans blue dye method.

ANALYSIS OF URINARY CALCULI THROUGH THE USE OF THE POLARIZING MICROSCOPE: A. Randall IV. Jr. Urol., vol. 48, No. 6, Dec., '42, p. 642.

The calculus is embedded in methyl methacrylate, commercially known as "Lucite" or "Plexiglass" and sections ground and stained. These are then examined under the polarizing microscope. Out of 58 calculi so studied, 4 components were identified; namely, calcium oxalate as the dihydrate and monohydrate, magnesium ammonium phosphate hexahydrate and uric acid or its salts. Pictures of these are given.

Small bits of the stone may be tested with a drop of 5% aqueous silver nitrate. A thin film of a canary yellow silver salt gradually darkening to brown indicates the position of phosphates. The murexide test may be used for uric acid and its salts.

SEASONAL SOMNOLENCE, A POSSIBLE POLLEN ALLERGY: L. Sternberg. Jr. Allergy, vol. 14, No. 1, Nov., '42, p. 89.

A case is reported in which the patient's only complaint was extreme drowsiness and mental dullness occurring repeatedly at the end of August and in September. On examination, the only significant physical findings were a systolic blood pressure of from 60-80 and an 8% eosinophilia. Skin tests were positive for ragweed pollen and for timothy.

As it was the close of the timothy season, treatment was limited to ragweed pollen with the patient comfortable except when overdosed. For the first time in 11 yrs. he was active every day. At the close of the pollen season his blood pressure was 130/80 and eosinophiles were 3%.

THE SEROLOGICAL AND BIOLOGICAL CLASSIFICATION OF HEMOLYTIC AND NONHEMOLYTIC STREPTOCOCCI FROM HUMAN SOURCES: L. A. Rantz. J. Inf. Dis., vol. 71, No. 1, July, '42, p. 61.

Streptococci isolated from various sources were transferred to blood agar and stored at 5°C until studied (usually 2 wks. or less). Antigens for the precipitin test were prepared by a modification of the formamide method of Fuller. Specific sera were obtained from Dr. Lancefield and from Lederle Laboratories. Only organisms from sources other than the respiratory tract were studied. Of 392 strains observed, 81.9% could be assigned to a Lancefield group. The various characteristics of the organisms observed are presented and discussed.

THE OCCURRENCE OF UNUSUAL SALMONELLA SPECIES: S. Bornstein & I. Saphra. Jr. Inf. Dis., vol. 71, No. 1, July, '42, p. 55.

Salmonella types previously reported only in animals should now be regarded as potential pathogens for man. They may present a clinical picture of enteric fever or even of a sepsis instead of the classical food poisoning symptoms.

New types isolated from human cases were: S. sendai, S. saint paul, S. eastbourne, S. worthington, S. maleagridis, S. urbana, S. san diego, S. manhattan and S. senftenberg.

BLOOD FINDINGS ON TWENTY PATIENTS BEFORE AND AFTER EXTRACTION OF TEETH: P. S. Faillo. J. Dent. Res., vol. 21, No. 1, Feb., '42, p. 19.

An immediate leucopenia lasting about 1 hr., followed by a leucocytosis was observed in most cases. Blood cultures were negative in all 11 cases studied immediately following extraction but became positive in intervals varying from 1-6 hrs. after extraction.

ROOT CANAL STUDIES. ANAEROBIC CULTURES: F. W. Morse & M. F. Yates. J. Dent. Res., vol. 21, No. 1, Feb., '42, p. 5.

Sodium thiogylcollate media were used for anaerobic root-canal cultures in an effort to increase the number of positive findings. As no greater number of positives resulted, the authors conclude that, on the basis of their work and all the work previously reported, the high percentage of negative cultures obtained from root canals is probably not due entirely to unsatisfactory methods of culture.

ON THE SITE OF FORMATION OF DIPHTHERIAL TOXIN: H. E. Morton & L. M. Gonzalez. Jr. Imm., vol. 45, No. 1, Sept., '42, p. 63.

Well washed cells of C. diphtheriae were broken up by sonic vibration and the extract obtained used for skin tests on rabbits. Normal animals gave positive reactions while those which had been immunized with antitoxin gave no reaction. Flocculation tests were also positive.

This would indicate that at least one site of formation of the exotoxin of C. diphtheriae is within the bacterial cell.

BOOK REVIEWS

WHAT THE CITIZEN SHOULD KNOW ABOUT WARTIME MEDICINE: By Lt. Col. Joseph R. Darnall, Medical Corps, United States Army, and V. I. Cooper. W. W. Norton & Company, Incorporated, 70 Fifth Ave., New York, 1942, Pp. 237. Price $2.50.

The authors have accomplished a worthy aim by describing wartime medical organization in a manner comprehensible to the average layman. Medical men would find the volume not only readable but informative as well. The intricacies of military medical service for a rapidly expanding Army and Navy and their bearing on the civil emergency are ably covered, with emphasis, of course, on the military aspect throughout.

Highlighting the book necessitates mention of the sections on the diversified activities of the medical services which embrace surgery, dentistry, sanitation, hygiene, communicable and other diseases. There is a closing note on the implications of demobilization from a retrospective as well as prophetic viewpoint. There are eleven illustrations, references by chapters and an adequate index.

BLOOD SUBSTITUTES AND BLOOD TRANSFUSION: Edited by Stuart Mudd, M.A., M.D., Professor of Bacteriology, University of Pennsylvania School of Medicine, Philadelphia and William Thalhimer, M.D., Director, Human Serum Division, Public Health Research Institute of the City of New York, Inc. Published by Charles C. Thomas, Springfield, Illinois and Baltimore, Maryland. Price $5.00 postpaid.

This symposium-monograph contains contributions of 70 authors and discussants and represents the communications of the annual meeting of the American Human Serum Association held in Cleveland on June 2 and 3, 1941.

The first section is devoted to the etiology and mechanisms of secondary shock. Interesting experimental observations are presented on blood volume and circulation in traumatic shock produced

in normal and sympathectomized dogs. The use of heat and cold in experimentally produced shock is evaluated. Theories and facts are presented in the chapter on the vascular and cellular dynamics of shock. There are many questions on this subject yet to be answered. A good working table is given showing the relationship of various factors in the pathologic physiology of shock.

The second section gives the methods of preservation of plasma and serum by desiccation and by freezing. Detailed diagrams, instructions and cost are given for the unit desiccator. A method for drying serum in vacuo from the frozen state in cellophane bags and other methods for drying and preserving serum and plasma are discussed in detail. Other chapters give the effect of the rate of thawing, clinical use and the electrophoretic patterns of serum and plasma.

The third and fourth sections are given over to blood substitutes, methods of preparation and preservation and a discussion by various medical officers of both the Army and Navy on their use under war conditions. There is a chapter on the Canadian project for the preparation of dried human serum for military use. Other chapters discuss the bacteriological control of plasma by various methods including the sulfone derivatives.

Section five takes up the storage, transportation, administration, isoagglutinins and blood group specific substances of whole blood. One chapter is given over to the antigenicity of the Rh blood factor in transfusion and pregnancy and its role in the etiology of erythroblastosis fetalis. A modified compatability test is suggested for the prevention of transfusion accidents. There is a good discussion on the neutralization of isoagglutinins anti-A and anti-B in O blood by means of the addition of the isolated blood group specific substances.

In section six the experimental and clinical experiences with the use of serum and plasma in the treatment of shock is given by a number of clinicians.

This symposium-monograph represents the pooling of knowledge, experiences and results of much experimental work of the many contributors in all phases of the use of plasma, serum, blood and substitutes in the treatment of shock. It is one of the most comprehensive reviews of the subject to date.

NEWS AND ANNOUNCEMENTS

NOTICE TO MEMBERS OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Owing to unusual circumstances existing this year in regard to holding the annual meeting, a ballot was taken from members of the Board of Directors and Officers of the Society for an opinion as to course to pursue. The following considerations were given due attention:

1. Transportation, being already overtaxed, may not be allowable by June.
2. Our members may be too busy to attend.
3. That it may not be patriotic or in keeping with the war effort. (The government has requested that conventions be discontinued.)

As a result of the ballot an unanimous decision was reached to postpone the annual meeting this year. Also, if necessary business warranted, that the officers and directors of the Society meet in Chicago to dispose of same.

We are in hope that we can announce further details in the next issue of the Journal regarding a plan whereby scientific papers which might have been presented at the meeting, if held, will be judged and awards for same announced.

WAR CONFERENCE

The medical, surgical and industrial hygiene experts who are so ably safeguarding the well-being of more than 20 million industrial workers have agreed to pool their knowledge and exchange their experiences regarding the many new and complex problems of today's wartime production. For this purpose their organizations—

The American Association of Industrial Physicians and Surgeons,

The American Industrial Hygiene Association, and

The National Conference of Governmental Hygienists—are combining their annual meetings in a four-day "War Conference"

at Rochester, New York, May 24-27, 1943. Among the problems to be discussed from a practical standpoint are:

The mass entry of women into industry;

Older-age employees, with their various associated problems; proper placement and employability considerations of the 4F rejectees;

Rehabilitation and proper employment of those already discharged from the military services because of disabling conditions;

Toxic and other hazards from new substances, new processes, and the use of substitute materials;

Absenteeism; fatigue; nutrition;

Effects of long hours; double shifts; two-job workers; overtime; increased industrial accident rates;

Advances in the treatment of illnesses and injuries, and many others.

This joint meeting will be a report on the state of the nation, by the men who know, in matters of industrial health. Dr. William A. Sawyer, Medical Director of Eastman Kodak, is General Chairman; Dr. James H. Sterner and Lieut. Comm. J. J. Bloomfield are arranging the programs for the industrial hygienists.

Physicians and surgeons, hygienists, engineers, nurses, executives—all who are interested in the problems of industrial health and their solution—are invited to attend as many of the sessions as they can arrange for; no registration fee is required.

ANATOMICAL CHARTS AND SKELETON CATALOGS

The Clay-Adams Company announces the publication of two new catalogs, the first, Catalog No. 154 on Human Anatomical Charts, twelve page catalog printed in two colors illustrating and describing a complete line of Anatomical Charts; the other, a four page, three color catalog describes human skeleton preparations and illustrates muscle skeletons, demonstration skulls, and other bone preparations. Catalogs will be sent on requests of teachers. Please use official letterhead.

Address inquiries to Clay-Adams Co. Inc., 44 East 23rd St., New York.

General expansion and intensification of the nation-wide fight against infantile paralysis, with progress made in scientific attacks on the crippling disease, are disclosed in the fourth annual report of

the National Foundation for Infantile Paralysis, issued today by Basil O'Connor, President of the Foundation.

The included financial statement reveals 77 grants and appropriations totaling $1,142,009.35 for the fiscal year ended September 30, 1942. The money was disbursed to medical schools, hospitals, research laboratories, health institutes and foundations, from funds raised by the "March of Dimes" and Celebrations of President Roosevelt's Birthday.

In addition to its national program, the Foundation also expanded its local activities. During the year 450 county chapters were formed, so that today 2,900 of America's 3,070 counties are serviced by approximately 40,000 volunteer workers.

With half of the subscription money returned to the counties of origin, the total return to the nation's local chapters since inception of the National Foundation in 1938 has reached the figure of $4,751,626.80, according to the report.

Highlights of the National Foundation's achievements during the past fiscal year were:

Teaching of the Kenny method of treating after-effects, which entailed establishment of special training programs to provide doctors, nurses and physiotherapists to apply and carry on the method.

Broadening of study in search of a prevention and real cure.

Addition not only to knowledge of the nature of the virus but also the method of spread of the infection.

Reemphasis of the little appreciated role of "spasm" and other temporary symptoms that if untreated result in permanent damage.

Correlation of the study of infantile paralysis with studies of related encephalitis infections.

The Committee on Virus Research of the National Foundation reported that continued studies gave further proof "that paralysis is but one symptom of the disease," that "paralysis may be looked upon as a complication of the disease" and that "added information indicated the possibility that all patients tested present evidence of poliomyelitis being an infection of the intestinal tract, and that the virus is routinely eliminated from the body through this tract."

Highly resistant to agents that kill ordinary bacteria, the virus

was shown to withstand the action of chlorine, phenol, sunlight, drying, ether and glycerine. Reported as possibly having been obtained in crystalline form, the virus was "so purified that material obtained from the brains and spinal cords of animals dying of infantile paralysis could be diluted many millions of times and still produce typical infection when injected into susceptible animals."

Research workers carrying on studies approved by the Committee on Research for the Prevention and Treatment of After-Effects demonstrated that in many human cases with apparent muscular paralysis, supposedly dead or involved nerves were "still capable of transmitting impulses," and, therefore, "present a hopeful prognosis if properly treated."

Work by electrical measurement methods further substantiated the repeated clinical observations that "there was 'spasm' of muscles and that this 'spasm' could be and was relieved by the Kenny method of treatment." It was also shown that "muscles presenting no manifest symptoms of involvement during the acute stage could likewise be in a state of 'spasm' which could persist if allowed to go untreated." This led to the belief that this unrecognized condition "has been responsible for the development of deformities in other than the obviously paralyzed parts long periods of time after the acute stage had passed."

Deformities due to contractures were shown to have occurred not only in wasted muscles "but also in muscles not before recognized as having been affected." The conclusion was that "there is hope of prevention of much of the curvature of the spine that constitutes such a large share of the crippling of infantile paralysis."

To make the Kenny method of treatment available to patients in all parts of the nation, the National Foundation enlarged its training course at the University of Minnesota, added six courses elsewhere and set up scholarships for training doctors, nurses and physiotherapists in this method.

The National Foundation through its Committee on Epidemics and Public Health reported that laboratory and field studies were carried on relative to the means of spread of St. Louis and equine encephalitis, which, like infantile paralysis, are virus diseases attacking the central nervous system. Not only was the method and spread of certain types of encephalitis "shown to be due to bites of insects" but means of prevention by vaccination "appear to be fairly cer-

tain." Methods developed for working with these diseases are being applied to the study of infantile paralysis.

For virus research the National Foundation made 23 grants to 21 institutions for a total of $543,749.46. Of the grants seven were to men or institutions whose work had not previously been supported by the Foundation.

Among the grants were: Johns Hopkins University School of Hygiene and Public Health, Baltimore, $59,244 (term of this grant is five years and the total approved for that period is $300,000); University of Michigan School of Public Health, Ann Arbor, $40,000; George Williams Hooper Foundation of the University of California, San Francisco, $21,526; Michigan Department of Health, East Lansing, two grants totaling $21,310; Yale University School of Medicine, New Haven, $16,000; University of Minnesota Medical School, two grants totaling $13,255.25; Children's Hospital Research Foundation of the University of Cincinnati College of Medicine, $12,400; University of Toronto Connaught Laboratories, Canada, $10,860; Standford University School of Medicine, San Francisco, $10,035; University of Southern California School of Medicine, Los Angeles, $10,000.

To carry on after-effects works, the Foundation made 23 grants to 16 institutions in 12 states and Canada, among them being 10 new grants. The total, including one appropriation, was $88,286.33. Among the grants were:

State University of Iowa College of Medicine, Iowa City, three grants totaling $11,893.38; University of Rochester School of Medicine and Dentistry, New York, $9,200; Massachusetts General Hospital, Boston, two grants totaling $7,975; University of Minnesota Medical School, Minneapolis, $7,050; University of Colorado School of Medicine, Denver, two grants totaling $7,000; and Columbia University College of Physicians and Surgeons, New York City, $5,900.

For educational purposes there were three appropriations and 12 grants to 10 institutions in 7 states, the total amount being $227,540.80. Principal grants were:

Teachers College of Columbia University, New York City, $6,000; National Organization for Public Health Nursing, Inc., New York City, three grants totaling $34,280; Georgia Warm Springs Foundation, $50,120; University of Minnesota Medical School, Minneapolis, $16,695; Northwestern University Medical School, Chi-

cago, $10,400; Harvard Infantile Paralysis Commission, Boston, $10,000; Stanford University School of Health (Women), Palo Alto, Cal., $9,520; American Physiotherapy Association, Palo Alto, Cal., $5,000; and D. T. Watson School of Physiotherapy, Leetsdale, Pa., $4,500.

For epidemics and public health work, there were five grants and eight appropriations, totaling $27,432.76. The grants were:

Illinois Committee on Infantile Paralysis in joint co-operation with Cook County Public Health Unit and Illinois Department of Public Health, Chicago, $8,950; New York State Department of Health, Albany, $3,600; Children's Hospital, Winnipeg, Canada, $1,080; Vanderbilt University School of Medicine, Nashville, Tenn., $940.72; Louisiana State University School of Medicine, New Orleans, $500.

Among the miscellaneous appropriations was $30,000 for operation of the infantile paralysis center at Tuskegee Institute, Alabama, which provides after-care for Negro orthopedic cases; also $225,000 to the Georgia Warm Springs Foundation to conduct its work and its research studies.

Minnesota

The Board of Directors and chairmen of the standing committees met with and at the home of Martha Strolberg, president of the Minnesota Society of Medical Technologists, on Friday, November 27, 1942, for the purpose of organizing the spring convention. Eleven members answered the roll call. The meeting started with a buffet luncheon at 6 p.m.

The following is a report made by Marion Polasky for publication in the *Minnesota Medical Technologist* on the continuation course offered at the Adult Center of the University of Minnesota: "Blood Banks and Blood Substitutes."

The first speaker gave the purpose and the use, as well as location, of bloodbanks for the Army, the Navy, the Red Cross; the importance of blood banks in hospitals and all over the country, especially those located in or near defense areas, so that blood would be available for the treatment of shock, burns, and various accidents.

The speaker gave suggestions on the preparation of a blood bank, namely refrigeration, procurement of donors, and complete histories of donor and patieht. Histories include name, age, serology, susceptibility to allergins, and tropical diseases.

There were descriptions of the various types of blood transfusion apparatus put up by commercial firms such as Upjohn, Abbott, Baxter, as well as descriptions of sets to be made in one's own laboratory.

Great stress was laid by each of the speakers on the scrupulous care with which the glassware and rubber tubing must be washed, sterilized, and packed so that there would be no chance of contamination.

There were talks on typing and crossmatching. It was suggested that the Landsteiner classification be used.

We were also told of some of the blood substitutes we may use in certain conditions during and after the war.

After each lecture, time was given for round table discussion.

We witnessed demonstrations of blood collecting, storing, and refrigeration at the University Hospital which were very helpful and informative. That served to drive home the fact that great care must be exercised in every step of the process.

On Saturday, January 9, 1943, at 10 a.m. in the Radisson Hotel, St. Paul, Minnesota, the President of the Minnesota Hospital Association met with the presidents and secretaries of hospital groups allied with the association. The medical technologists were represented by their state president, Martha Strolberg, and state secretary, Esther Wilbrecht. The meeting was directed toward streamlining the annual convention program and narrowing it down to cover only two instead of three days. A pro rata group fee of $5.00 was voted to displace the personal registration fee of 50c per individual. Minneapolis will very likely be the convention center and the date some time in May of this year.

The Minnesota Medical Technologists are proud to think that one of their number was elected at large to represent them at the meetings of the Advisory Board which meets in conjunction with the Pathologists on the Board of Registry for Medical Technologists. There are five members on this board whose function is to serve as the link between the technologists and pathologists in matters pertaining to the medical technologists. The other four are two officers of the National Society of Medical Technologists and two are elected from the registrants as appearing on the Roster of Medical Technologists of the American Society of Clinical Pathologists.

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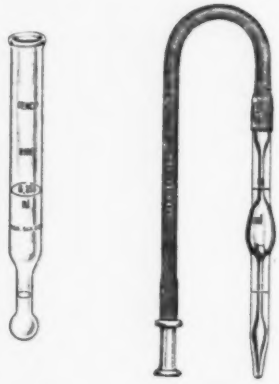

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